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Sensitive and Rapid Molecular Detection Assays for *Salmonella enterica* Serovars Typhimurium and Heidelberg†

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ABSTRACT

Salmonella enterica is a significant cause of gastroenteritis worldwide, with serovars Typhimurium and Heidelberg being particularly prevalent, which have broad host ranges infecting poultry, dairy animals, and humans. Traditional methods used for the detection of *Salmonella* from contaminated food products are time-consuming and labor-intensive. The aim of this study was to develop a sensitive and rapid PCR-based detection method with optimized specificity for high-throughput screening of food and clinical samples. We used bioinformatics to identify potential serovar-specific regions from the available *S. enterica* sequenced genomes. We designed primer pairs to targeted regions unique to Typhimurium and Heidelberg. A primer pair targeting a putative cytoplasmic protein STM4492 amplified a 759-bp product specific to Typhimurium, and a primer pair targeting a putative inner membrane protein STM2745 amplified a 199-bp product from both Typhimurium and Heidelberg. A primer pair for the *oriC* locus was used to identify all *Salmonella*. We screened 217 isolates including the *Salmonella* reference collections A and B, validating the specificity of each primer set. Next, a multiplex PCR (mPCR) assay and quantitative real-time PCR assay were optimized for identification and differentiation of Typhimurium and Heidelberg. An mPCR assay was developed and successfully detected *S. enterica* isolates from inoculated Cheddar cheese, raw turkey, and cooked turkey at concentrations as low as 1 CFU/g of food. The reaction conditions for this mPCR have significantly reduced the time needed to identify *S. enterica* Typhimurium and Heidelberg, making this a rapid selective tool.

Salmonellosis is one of the most common infectious foodborne diseases in the world, both in animals and humans. *Salmonella enterica* can cause a wide spectrum of illnesses, ranging from gastroenteritis to severe, life-threatening enteric fever. Each year, an estimated 1.4 million human cases of salmonellosis occur in the United States *(25)*. *S. enterica* was the second most common cause of human zoonosis reported in 2006 throughout the European Union, with a total of 160,049 confirmed cases of salmonellosis (34.6 cases per 100,000 people). *S. enterica* infections are a significant source of gastroenteritis in Ireland, with 1,100 isolates having been reported to the National *Salmonella* Reference Laboratory in 2007. Traditional detection methods for *Salmonella* are culture based and can take 4 to 7 days to confirm a positive sample. Due to the health risks posed by *Salmonella* infections, detection must be rapid, sensitive, and accurate to reduce false-negative and -positive results.

S. enterica Typhimurium is one of the main causes of gastrointestinal illness globally *(9, 25)*. In 2007, the National *Salmonella* Reference Laboratory found that *S. enterica* Typhimurium was the second most common serovar isolated, accounting for 25% (114 isolates) of all *Salmonella* cases from human sources in Ireland. Of the nonhuman isolates typed, *S. enterica* Typhimurium accounted for 38% (246) *(1)*. *S. enterica* Heidelberg and Agona each accounted for 0.5% (3) of the total number of isolates typed from human sources in 2007. *S. enterica* Typhimurium is one of the top three serovars isolated from beef carcasses, ground beef, pig carcasses, and ground pork *(34)*. *S. enterica* Heidelberg was found to be the predominant serovar isolated from both chicken and turkey carcasses and ground raw meat *(19, 28–30)*.

To date, there are five complete whole-genome sequences from the genus *Salmonella* published, belonging to four different serovars *(4, 7, 22, 23, 33)*. Recent studies carried out by Kim and colleagues *(16–18)* have focused on the published *S. enterica* Typhimurium LT2 genome, and have identified potential serovar-specific regions that could be used to differentiate *S. enterica* Typhimurium isolates from other *Salmonella* serovars.

In this study, we performed in silico genome comparisons of available sequenced *Salmonella* genomes to identify potential serovar-specific regions for Typhimurium and Heidelberg. These regions were used to develop specific PCR assays for rapid detection and identification of serovars

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Typhimurium and Heidelberg. Multiplex PCR assays were then validated for sensitivity and specificity at detecting these serovars in different food matrices. Finally, a quantitative real-time PCR (qRT-PCR) method was developed, resulting in increased speed and sensitivity over conventional PCR methods.

MATERIALS AND METHODS

Bacterial strains. A total of 217 isolates were used to validate the techniques developed during this investigation. In order to investigate the specificity of potential serovar regions identified from in silico genome comparisons, two reference collections were used in this study: *Salmonella* reference collection A (consisting of 72 isolates of serovars from the *S. enterica* Typhimurium complex) and *Salmonella* reference collection B (composed of 72 strains, encompassing 37 serovars of *S. enterica* subsp. I) *(2, 3)*. Both collections represent a geographically diverse panel of strains, encompassing an extensive selection of various serovars. In addition, 73 recent clinical and environmental Irish isolates were examined, which encompassed 28 different serovars (Table 1). As negative controls, three National Collection Type Culture (NCTC) strains were also included in this study: *Escherichia coli* NCTC 9009, *Listeria monocytogenes* NCTC 11994, and *Staphylococcus aureus* NCTC 6571. DNA was isolated from each strain by using the Gnome DNA isolation kit (BIO 101, Inc., La Jolla, CA) and/or the Promega (Madison, WI) Wizard genomic DNA purification kit according to the manufacturer's instructions.

Genome analysis. At the inception of this project, five fully sequenced, published *S. enterica* genomes for serovars Typhimurium LT2, Choleraesuis ATCC SC-B67, Typhi CT18, Typhi Ty2, and Paratyphi A were available in the database, and 20 *Salmonella* genomes were in progress. The complete nucleotide sequences and annotations for each were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/Genomes/). A bioinformatic approach was utilized to detect and identify potential serovar-specific regions for serovars Typhimurium and Heidelberg. Whole linear genome comparisons were carried out with WebACT (http://www.sanger.ac.uk/Software/ACT/). Basic Local Alignment Search Tool (BLAST) searches were performed to further interrogate regions (http://www.ncbi.nlm.nih.gov/COG/).

Primer pair design and PCR conditions. Primers were designed to amplify potential serovar-specific regions identified by comparative genomics, and a primer set specific to the *oriC* locus was used as a *Salmonella*-specific probe and internal control *(37)* (Table 2). PCR reactions were carried out with the following reagents from the Promega GoTaq PCR kit: 5 μl of 5× GoTaq Flexi Buffer, 1 μl of 25 mM $MgCl_2$, 1 μl of 1.25 mM mix of each nucleotide, 1.5 μl of each primer at 10 pmol/μl, 1 μl of DNA polymerase (5 U/μl), DNA template, and sterile water, to a 25-μl total volume. PCR amplification was performed in an MJ Thermal Cycler-2000 (BioRad, Hercules, CA), with an initial denaturation of 96°C for 2 min, 94°C for 30 s, annealing temperature according to each primer set for 30 s, and 72°C for 45 s for 30 cycles. A multiplex PCR (mPCR) reaction was designed by using a three–primer pair set for *oriC*, STM4492, and STM2745. PCR amplification was performed in an MJ Thermal Cycler-2000, with an initial denaturation of 96°C for 2 min, 94°C for 30 s, annealing temperature of 58°C for 30 s, 72°C for 45 s for 19 cycles, and a final extension at 72°C for 10 min. PCR products were visualized on 0.8% (wt/vol) agarose 1× Tris-acetate-EDTA buffer gels stained with ethidium bromide (0.5 μg/ml) by using a UV transilluminator (BioRad).

Validation of mPCR in food matrices. The ability of our mPCR to detect and identify *S. enterica* in food samples was investigated. Turkey meat was chosen, as it is low in fat and sodium, and it has emerged as an alternative to red meat as part of a healthy lifestyle. In addition, *S. enterica* Typhimurium and Heidelberg were found to be among the top six *S. enterica* serovars isolated from raw ground turkey meat in the United States *(34)*. The European Food Safety Authority found that 30.7% of all fattening turkey flocks were positive for *Salmonella,* including isolates of *S. enterica* Typhimurium and Heidelberg *(10)*. Foods that have high fat content have been found to have a lower infective dose *(6)*. We chose Cheddar cheese as an example of a high-fat food matrix to be examined.

To determine to what extent the mPCR assay could accurately detect the concentration of bacterial standards added to matrices, the following presumptive-free matrices were seeded in triplicate: buffered peptone water (BPW; Merck, Darmstadt, Germany), Rappaport-Vassiliadis (RVS; Merck), turkey (raw and cooked), and Cheddar cheese samples. Current culture-based detection methods were utilized, and the preselective and selective broths were analyzed by mPCR. The International Organization for Standardization (ISO) Standard 6579 and Health Protection Agency culture-based methods to detect *Salmonella* were used throughout this study. A set of 10-fold serial dilutions were prepared in maximum recovery diluent (Merck) from an overnight culture of *Salmonella,* and a plate count was performed in duplicate by using xylose-lysine-deoxycholate agar (Merck) incubated at 37°C for 24 h. To each 25-g sample matrix, 1 ml of each dilution (neat through 10^{-10}) and 225 ml of sterile BPW (containing 1.6 ml of 0.1% novobiocin) broth were added. The sample was homogenized with a stomacher (Seward, London, UK) for 60 s and incubated at 37°C for 18 h. Controls were set up for each matrix by using 1 ml of nutrient broth. Selective enrichment of the sample consisted of adding 100 μl of BPW enrichment to 9.9 ml of RVS broth incubated at 41.5°C for 24 h. The selective enrichment broth RVS was treated according to the method of dos Santos *(8),* with minor modifications. A 5-μl aliquot was added to each mPCR reaction as template. The specificity of the assay was assessed by spiking samples with overnight cultures of *S. enterica* serovars Dublin, Agona, and Enteritidis, *E. coli, L. monocytogenes,* and *S. aureus,* and performing PCR assays. PCR products were separated by electrophoresis on a 0.8% agarose gel in 1× Tris-acetate-EDTA buffer, and visualized with ethidium bromide and UV transillumination. Presence of products of the appropriate size was visually verified by comparison to DNA molecular weight standards (1- and 10-kb markers; New England Biolabs, Ipswich, MA).

qRT-PCR. qRT-PCR primers and probes were designed by using the same target genes as in the mPCR assay. The primer sets were redesigned to yield smaller amplicons suitable to qRT-PCR systems (Table 2). Sequence-specific primers and TaqMan probes were designed by using Primer3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Dyes were chosen with specification for Rotor-Gene 6000 (Corbett Research Biosciences, Sydney, Australia). The dyes chosen for this study were FAM (470–510 nm), Cy5 (625–660 nm), and ROX (585–610 nm), and the quenchers used were BHQ1, BHQ2, and BHQ3. qRT-PCR was performed by using the QuantiTect multiplex PCR NoRox kit, following the manufacturer's instructions (Qiagen, Valencia, CA). Amplification conditions used were as follows:

TABLE 1. *List of* Salmonella enterica *strains isolated in Ireland screened in this study*

Salmonella serovar	No. of strains	Source (*n*)[a]	Yr isolated (*n*)[b]
Agona	12	Cork County Council (12)	2002 (1), 2005 (11)
Bareilly	1	Reference Laboratory, Galway (1)	—[c]
Brandenburg	1	Reference Laboratory, Galway (1)	—
Bredeney	2	National Food Center, Dublin (1)	—
		Reference Laboratory, Galway (1)	
Choleraesuis	1	Taiwan (1)	—
Derby	2	UCG Microbiology Laboratory (2)[d]	—
Dublin	9	Cork County Council (9)	2001(1), 2002(3), 2003(5)
Enteritidis	5	National Food Center, Dublin (1)	—
		UCG Microbiology Laboratory (4)	
Give	1	Reference Laboratory, Galway (1)	—
Gold Coast	1	Reference Laboratory, Galway (1)	—
Hadar	1	UCG Microbiology Laboratory (1)	—
Heidelberg	1	Reference Laboratory, Galway (1)	—
Indiana	1	Reference Laboratory, Galway (1)	—
Infantis	2	UCG Microbiology laboratory (2)	—
Java	1	Reference Laboratory, Galway (1)	—
Kedougou	1	Reference Laboratory, Galway (1)	—
Kentucky	4	National Food Center, Dublin (1)	2002 (2)
		Reference Laboratory, Galway (1)	
		Cork County Council (2)	
Kottbus	1	Reference Laboratory, Galway (1)	—
Montevideo	1	UCG Microbiology Laboratory (1)	—
Panama	1	Reference Laboratory, Galway (1)	—
Poona	1	UCG Microbiology Laboratory (1)	—
Reading	1	UCG Microbiology Laboratory (1)	—
St. Paul	2	UCG Microbiology Laboratory (2)	—
Stanley	1	Reference Laboratory, Galway (1)	—
Stanleyville	1	Reference Laboratory, Galway (1)	—
Thompson	2	UCG Microbiology Laboratory (2)	—
Typhimurium	14	Cork County Council (14)	2000 (2), 2001 (4)
			2002 (4), 2003 (4)
Virchow	2	National Food Center, Dublin (1)	—
		UCG Microbiology Laboratory (1)	—

[a] Number of strains from this source.
[b] Number of isolates from this year.
[c] —, information not available.
[d] UCG, National University of Ireland, Galway.

initial denaturation 95°C for 15 min, which was followed by 95°C for 45 s, and 60°C for 75 s for 30 cycles. A standard curve was prepared by using genomic DNA from Typhimurium LT2. The copy number was determined by using the formula: $m = (n)(1.096 \times 10^{-21}$ g/bp), where m is the mass of the DNA, and n is the number of base pairs. The optical density of the DNA was determined by using a NanoDrop (Thermo Scientific, Wilmington, DE), and a series of dilutions was performed in duplicate to yield aliquots of specific, known copy numbers. The reaction was carried out with the cycling conditions outlined above, and a threshold value for each channel was assigned by Rotor-Gene software (Corbett Research, Mortlake, New South Wales, Australia).

The sensitivity of this qRT-PCR assay was assessed by spiking ground turkey samples with various dilutions of *S. enterica* Typhimurium cells. Samples were also spiked with *S. enterica* Heidelberg, Dublin, and Enteritidis cells. Serial dilutions of each strain were prepared, and 0.1 ml was plated onto two xylose-lysine-deoxycholate plates per dilution and incubated at 37°C to enumerate the seeded concentration.

Both conventional and qRT-PCR assays were performed from the preselective and selective enrichment steps, BPW and RVS, respectively. One microliter of the broth was used as template for qRT-PCR. All enrichments were in accordance with ISO detection methods. A nonspiked sample was also tested, and a PCR no-template control was used.

RESULTS

mPCR assay. Whole-genome sequences from the five published *S. enterica* strains were obtained from the National Center for Biotechnology Information Web site, and genomic comparison analysis was performed by using WebACT (www.webact.org). Unique regions were identified, and BLAST searches were performed against *Salmonella* genome sequences in progress. From these analyses, four regions were identified as serovar specific, and primer pairs were designed to each a gene within each region: STM2234 (putative phage tail fiber), STM3291 (putative cytoplasmic protein), STM2745 (putative inner membrane

TABLE 2. *Details of all the primers and probes designed and used in the multiplex PCR*

Specific target	Primer name	Sequence	Product size (bp)
Conventional PCR			
Salmonella Typhimurium and Heidelberg	STM2745-F	5′-CGGTCTGACCAATATCTCCA-3′	199
	STM2745-R	5′-GCCACCAGTCAGGTAGTGG-3′	
Salmonella Typhimurium	STM4492-F	5′-ACAGCTTGGCCTACGCGAG-3′	759
	STM4492-R	5′-AGCAACCGTTCGGCCTGAC-3′	
Salmonella serovars	ConOri-F	5′-GCGGTGGATTCTACTCAAC-3′	461
	ConOri-R	5′-AGAAGCGGAACTGAAAGGC-3′	
qRT-PCR			
Salmonella Typhimurium and Heidelberg	RT-STM2745-F	5′-TCACCTGCGACAGCCATGA-3′	99
	RT-STM2745-R	5′-TGAGCATCGCCATCGGCAT-3′	
	RT-STM2745-Pr	5′-CAGCTATGGCCGACTGCTGGAAT-3′ FAM-ATTCCAGCAGTCGGCCATAGCTG-BHQ1 (reverse complement)	
Salmonella Typhimurium	RT-STM4492-F	5′-CATTGATGCCATGGGTGACA-3′	133
	RT-STM4492-R	5′-CGTGACGATAATCCGTGTAC-3′	
	RT-STM4492-Pr	5′-ACTGAAGGATTTAGTGACTCGTGTA-3′ Cy5-TACACGAGTCACTAAATCCTTCAGT-BHQ3 (reverse complement)	
Salmonella spp.	RT-PCRconori-F	5′-GGATCGCACGATCTTTACACT-3′	86
	RT-PCRconori-R	5′-GAACATGAGATTCCGGAAGAT-3′	
	RTConOri-Pr	5′-AATCCAGGATCCGAGCCAAATCTC-3′ Rox-AATCCAGGATCCGAGCCAAATCTC-BHQ2	

protein), and STM4492 (putative cytoplasmic protein). PCR assays were used to screen the collection of 217 *Salmonella* strains, encompassing 48 serovars. A primer pair designed to target the origin of replication (*oriC*) was used as a *Salmonella* species internal control, which yields a 461-bp product specific to all *Salmonella* serovars *(37)*. Two primer pairs were identified as being serovar specific, STM4492F and STM4492R, which yielded a 759-bp product specific to *S. enterica* Typhimurium only, and STM 2745F and STM 2745R, which yielded a 199-bp product specific to *S. enterica* Typhimurium and Heidelberg only.

An mPCR assay was developed by using the three primer pair sets for STM4492, STM2745, and the *oriC* locus to differentiate serovars Typhimurium and Heidelberg from all other salmonellae. The mPCR assay screened 217 salmonellae and identified all *Salmonella* strains tested by yielding a single *oriC*-specific band of 461 bp, whereas only Typhimurium isolates yielded the expected three PCR bands of 199, 461, and 759 bp, and only Heidelberg isolates yielded two PCR bands of 199 and 461 bp (Fig. 1A). The specificity and sensitivity of the mPCR assay was investigated further by seeding food matrices with *S. enterica* serovars Typhimurium, Heidelberg, Dublin, Agona, and Enteritidis; *E. coli, L. monocytogenes,* and *S. aureus* at a level of 10^5 CFU. Serovars Typhimurium and Heidelberg yielded bands of the expected sizes, and serovars Dublin, Agona, and Enteritidis yielded only a single specific PCR product of 461 bp (Fig. 1B). *E. coli, L. monocytogenes,* and *S. aureus* did not yield any amplicons (Fig. 1B).

In order to validate our mPCR assay further as a detection method for *Salmonella,* we tested spiked food matrices as outlined in ISO Standard 6579 and in the Health Protection Agency–based method. Prior to mPCR assays, food samples and preselective (BPW) and selective broth (RVS) matrices were seeded with LT2, as outlined in ''Materials and Methods.'' Serial dilutions of these were used to determine detection limits in BPW and RVS. These were 780 CFU for BPW (Fig. 2A) and ≤1 CFU for RVS (Fig. 2B). Ground turkey (both raw and cooked) and grated-cheese samples were seeded with serial dilutions *S. enterica* Typhimurium LT2 cells. DNA from Typhimurium strain LT2 and Heidelberg were used as positive controls, while broth samples that were not seeded with bacterial cells were used as negative controls. All assays were performed in triplicate on at least two separate occasions. The detection limit for the grated-cheese sample was found to be 7 CFU/25 g of sample (Fig. 3A). The sensitivity in raw ground turkey was found to be 1,200 CFU/25 g of sample (Fig. 3B). Greater assay sensitivity was obtained when cooked turkey samples were seeded with *S. enterica* Typhimurium LT2 cells with a limit of detection of ≤1 CFU/25 g of sample (Fig. 3C).

qRT-PCR. TaqMan probes were designed; FAM dye was used to detect STM2745, Cy5 dye was used to target STM4492, and Rox dye was used to detect *oriC* (Table 2). A standard curve was prepared by using Typhimurium LT2 genomic DNA. Each sample was analyzed in duplicate, and threshold values for each channel were assigned by the Rotor-Gene software. DNA samples of common *Salmonella* serovars were investigated. The correlation coefficient (R^2) value was calculated and used to compare the data points observed to the standard curve points expected; the closer the R^2 value was to 1, the better the fit of the data to the

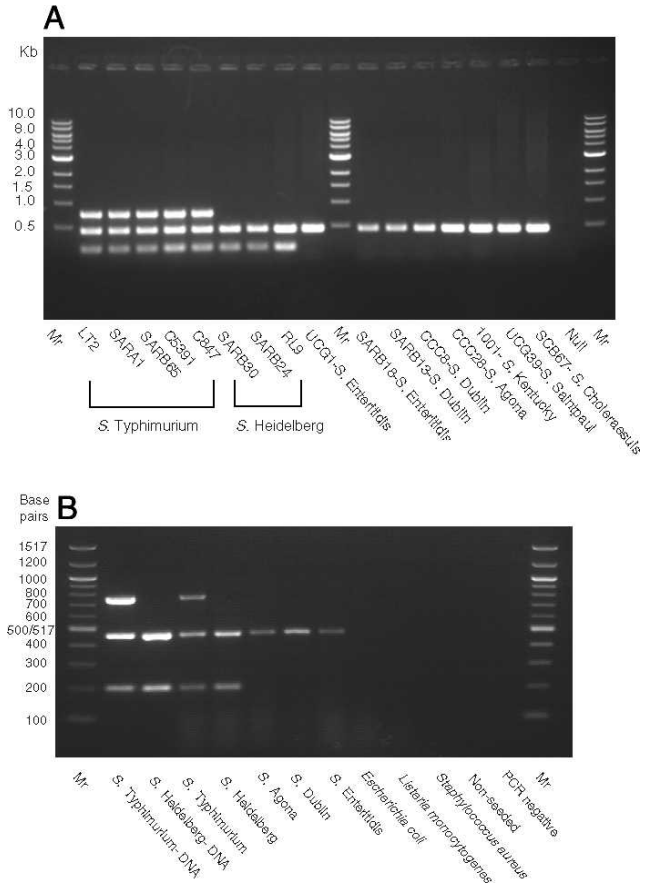

FIGURE 1. *mPCR assays. (A) mPCR assay of a representative panel of* Salmonella *isolates. Typhimurium strains yielded three bands, Heidelberg isolates yielded the expected two bands, and all* Salmonella *serovars yielded the expected 461-bp band. (B) mPCR assay of food matrices seeded with* Salmonella *serovars Dublin, Agona, and Enteritidis,* Escherichia coli, Listeria monocytogenes, *and* Staphylococcus aureus. *DNA from* Salmonella enterica *Typhimurium LT2 and Heidelberg were used as positive controls yielded the expected three PCR bands and two PCR bands, respectively.*

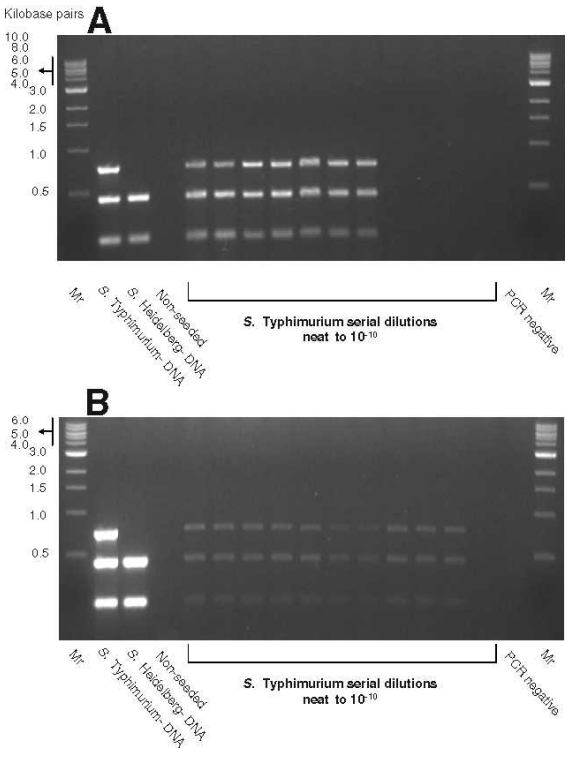

FIGURE 2. *mPCR assay. (A) Aliquots of BPW broth were seeded with various concentrations of* Salmonella enterica *Typhimurium LT2 cells. A detection limit of 780 CFU was achieved. (B) Aliquots of RVS broth were seeded with various concentrations of* S. enterica *Typhimurium LT2 cells. A detection limit of ≤1 CFU was achieved.*

expected standard curve line. Our mPCR reaction yielded R^2 values >0.99. The slope of the standard curve is directly related to the average efficiency of amplification and may be used to calculate the PCR efficiency. A 100% efficient reaction should yield a slope of −3.32 *(12)*. The slope values for this qRT-PCR reaction range from −3.32 to −3.53. The number of qRT-PCR cycles required to detect control strains were also calculated, and ranged from a cycle threshold (Ct) value of 13.41 to 18.12.

Both conventional mPCR and qRT-PCR assays were carried out at the preenrichment (BPW) and selective enrichment (RVS) stages *(24)*. The mPCR assay was validated with ground raw turkey samples seeded with *S. enterica* Heidelberg, Dublin, and Enteritidis cells and by using various concentrations of Typhimurium. The initial seeding levels for the meat samples were as follows: 6.1×10^8 *S. enterica* Typhimurium LT2/ml, 5.6×10^9 *S. enterica* Heidelberg (RL9)/ml, 7.5×10^8 *S. enterica* Dublin (CCC8)/ml, and 8.8×10^8 *S. enterica* Enteritidis (UCG1)/ml. DNA from LT2 and RL9 was used as positive control samples. As shown in Table 3, the qRT-PCR reaction was more sensitive than was the conventional mPCR assay from both broths tested for the detection of serovar Typhimurium (Table 3). Using the BPW broth, the detection limit for *S. enterica* Typhimurium from the conventional PCR was 6.1×10^6 CFU/ml, whereas the corresponding qRT-PCR analysis yielded a detection limit of 6.1×10^3 CFU/ml (Table 3). Using the RVS broth, the detection limit for *S. enterica* Typhimurium from the conventional PCR was 6.1×10^3 CFU/ml, whereas the corresponding qRT-PCR yielded a detection limit of 6.1×10^1 CFU/ml (Table 3). Two negative controls were used, which consisted of food matrix without any bacterial cells added and a PCR reaction with dH_2O instead of template DNA. All positive controls yielded expected bands in both the conventional and qRT-mPCR from both BPW and RVS (Table 3). The negative controls did not yield any amplicons in the conventional and RT-PCR assays from either BPW or RVS (Table 3).

DISCUSSION

Molecular-based detection methods have emerged as rapid and reliable ways to detect *S. enterica*, and various loci have been used as target genes including *invA, fimA, sdiA, sefA,* and *fliC (5, 13, 27, 35, 36)*. In this study, we used bioinformatics to identify two regions specific to serovars Typhimurium and Heidelberg, and a total of 217 *S. enterica* strains were screened with a primer pair targeting a putative

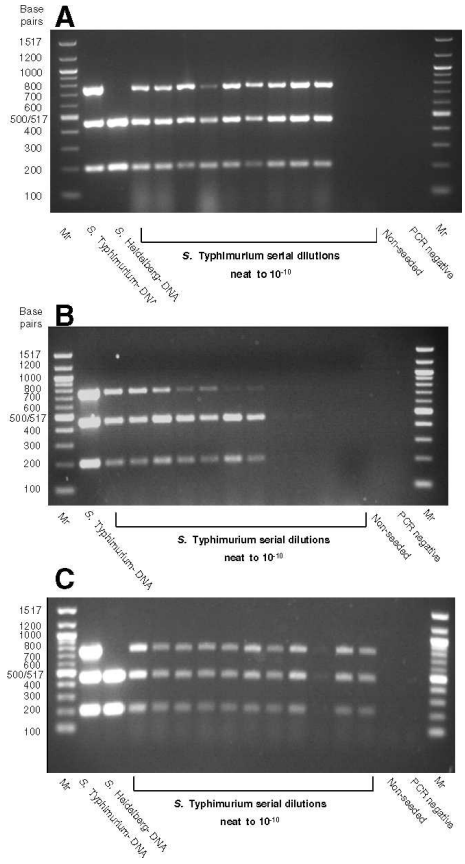

FIGURE 3. *mPCR assay. (A) Grated-cheese samples were seeded with various concentrations of* Salmonella enterica *Typhimurium LT2 cells prepared by serial dilution.* Salmonella *cells were detected down to a* 10^{-8} *dilution, which corresponded to a detection limit of 7 CFU. (B) Raw ground turkey meat samples were seeded with various concentrations of* S. enterica *Typhimurium LT2 cells prepared by serial dilution.* Salmonella *cells were detected down to a* 10^{-6} *dilution, which corresponded to a detection limit of 1,200 CFU. (C) Cooked ground turkey meat samples were seeded with various concentrations of* S. enterica *Typhimurium LT2 cells prepared by serial dilution.* Salmonella *cells were detected down to a* 10^{-10} *dilution, which corresponded to a detection limit of* $\leq$*1 CFU.*

inner membrane protein (STM2745), another primer pair targeting a putative inner membrane protein (STM4492), and finally, a third primer pair that targeted the *oriC* locus present in all *Salmonella (37)*. Our mPCR assay using these primers specifically detected and differentiated between *S. enterica* Typhimurium and Heidelberg, and was validated for sensitivity and specificity (Fig. 1). The cycling conditions for the mPCR assay involved 20 cycles and took 75 min to complete, making it a very rapid method for the detection of *Salmonella*.

We demonstrated the specificity of our mPCR assay in artificially spiked incurred samples, broth media, Cheddar cheese, and raw and cooked ground turkey meat seeded with serovars Dublin, Agona, and Enteritidis and foodborne pathogens *E. coli, L. monocytogenes,* and *S. aureus*. *Salmonella*-specific amplicons were found in all *Salmonella* strains screened. No amplicons were observed in the non-*Salmonella* strains (Fig. 1).

The sensitivity of our mPCR assay was shown by seeding a number of different matrices with serial dilutions. The sensitivity of the assay from both preselective (BPW) and selective (RVS) enrichment broths without any additional sample matrix was determined to be 780 CFU/ml from BPW and $\leq$1 CFU/ml from RVS (Fig. 2). Oliveira and colleagues *(32)* compared the use of selective and nonselective enrichment broths for PCR procedures, and demonstrated that RVS was more sensitive than were nonselective and standard microbiological techniques (SMT) methods. However, it was found that PCR from RVS broth detected more positive samples of *Salmonella* than did PCR from nonselective broth and SMT *(31, 32)*. Myint and coworkers *(26)* found that a preenrichment step was necessary to detect *Salmonella* by PCR in raw poultry samples, at a limit of 100 CFU/ml of selective broth–enriched sample. They found that PCR from BPW was 85% sensitive; after selective enrichment, in either of the *Salmonella*-selective broths RVS or tetrathionate Hajna broth, the sensitivity increased to 100%. The use of RVS as a template for our mPCR assay required a number of preparation steps *(8)*. The additional preparation steps for the mPCR assay developed in this study have added an additional 2 h to the protocol. However, the sensitivity increased from 780 CFU/ml in BPW to $\leq$1 CFU/ml in RVS samples.

We investigated the ability of our mPCR assay to detect *Salmonella* in various matrices, and found the limits of detection obtained for *Salmonella* in cheese, raw turkey, and cooked turkey samples were 7, 1,200, and $\leq$1 CFU/ml of selective enrichment, respectively. A study by Jeniokova and colleagues *(15)* investigated the effect of the matrix on a PCR assay using an immunomagnetic separation technique to extract the DNA, and found the presence of food sample debris in enrichment broths can be inhibitory to PCR. Interestingly, they found that high-fat-content samples such as soft cheese yielded a higher detection limit than did other lower-fat-containing foods such as eggs, with detection limits of 1.5×10^3 CFU/25 g of cheese sample and 1 to 5 CFU/25 g of egg sample. This is in contrast to our findings that the detection of *Salmonella* in a high-fat-content food such as grated cheese was more sensitive than in raw ground turkey meat. The matrix that seemed to exert the most negative effect on sensitivity was raw turkey meat. The reason for the reduced limit of detection in this matrix could have been due to the presence of inhibitory substances or competitive microbiological flora in the matrix.

TaqMan PCR assays have been developed for a number of common gene targets to detect *Salmonella* such as the *stn* gene, the *ttrRSBCA* locus, and the *invA* gene *(14, 20, 21)*. In this study, novel qRT-PCR targets were designed to identify *Salmonella,* as well as differentiate between *S. enterica* Typhimurium and Heidelberg to a detection limit of 6.0×10^1 CFU/ml from RVS broth in less than 48 h. This is far more rapid than the ISO SMT method is for the detection of *Salmonella,* which can take 4 to 7 days to complete. Further analysis of *Salmonella* detected by this assay would still require the completion of the full ISO SMT method to yield a live culture; however, these can easily be done in parallel with any qRT-PCR–negative samples being discarded. This presents a significant savings in terms of time and labor. The

TABLE 3. *Conventional mPCR versus qRT-PCR for the detection of* Salmonella *isolates from raw ground turkey*

Salmonella enterica isolate	BPW						RVS					
	Conventional mPCR			qRT-PCR			Conventional mPCR			qRT-PCR		
	STM2745	STM4492	ConOri	STM2745	STM4492	ConOri	STM2745	STM4492	ConOri	STM2745	STM4492	ConOri
Typhimurium LT2 (DNA)[a]	+	+	+	+	+	+	+	+	+	+	+	+
Heidelberg RL9 (DNA)	+	−	+	+	−	+	+	−	+	+	−	+
No-template control	−	−	−	−	−	−	−	−	−	−	−	−
Typhimurium LT2 (neat)	+	+	+	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-1})	+	+	+	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-2})	+	+	+	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-3})	−	−	−	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-4})	−	−	−	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-5})	−	−	−	+	+	+	+	+	+	+	+	+
Typhimurium LT2 (10^{-6})	−	−	−	+	−	+	−	−	−	+	+	+
Typhimurium LT2 (10^{-7})	−	−	−	+	−	+	−	−	−	+	+	+
Typhimurium LT2 (10^{-8})	−	−	−	−	−	−	−	−	−	−	−	+
Typhimurium LT2 (10^{-9})	−	−	−	−	−	−	−	−	−	−	−	+
Typhimurium LT2 (10^{-10})	−	−	−	−	−	−	−	−	−	−	−	+
Heidelberg RL9	+	−	+	+	−	+	+	−	+	+	−	+
Enteritidis UCG1	−	−	+	−	−	+	−	−	+	−	−	+
Dublin CCC8	−	−	+	−	−	+	−	−	+	−	−	+
Null reaction[b]	−	−	−	−	−	−	−	−	−	−	−	−

[a] DNA is the positive control.
[b] Null reaction, nonspiked samples.

qRT-PCR assay designed in this study was found to be more sensitive than was the conventional mPCR assay from the preselective and selective enrichment broths, as previously shown by others *(11)*. qRT-PCR from nonselective broth (BPW) was less sensitive than was qRT-PCR from selective broth (RVS), with a detection limit of 6.1×10^3 CFU/ml from BPW and 6.1×10^1 CFU/ml from RVS.

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